binding and provided a dynamical basis for the efficacy of partial agonists or full agonists, as well as the modulation of activation by positive allosteric modulators (PAMs). Finally, our work provides a general model for the activation process of mGluRs.

1757-Plat
Biased Agonism at Opioid Receptors: Insights from Analysis of Structural Interaction Fingerprints
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Biased agonism, that is the ligand-dependent selectivity for specific signaling pathways in a G protein coupled receptor (GPCR), is an emerging, promising strategy for the development of safer drugs. In the case of the kappa opioid receptor (KOPr), its preferential activation of G-protein versus β-arrestin has been proposed to provide a more direct route to discovering non-addictive opioid therapeutics with reduced side effects. In fact, the KOPr-mediated dysphoria that usually accompanies the beneficial analgesic effect of KOPr agonists has recently been attributed to the activation of the p38 mitogen-activated protein kinase pathway, which is believed to follow arrestin recruitment to the activated KOPr. This observation suggests that KOPr agonists that selectively activate the G protein, but do not recruit arrestin, may be more effective analgesics since they would not exhibit the adverse effects triggered by the arrestin pathway.
Based on the above, understanding the structural and chemical determinants of biased agonism at the KOPr is highly desirable as it can guide the discovery/design of improved therapeutics. Here, we employ flexible docking of a set of recently characterized functionally selective KOPr ligands to establish a predictive model for G protein-biased agonism at this receptor based on characteristics of modes of interaction between the ligand and the KOPr. We apply the resulting classifier to a large set of established KOPr agonists to assess their ability to preferentially promote G protein coupling, arrestin recruitment, or both.

1758-Plat
Conformational Dynamics of a G Protein-Coupled Receptor at the Single-Molecule Level
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G protein-coupled receptors (GPCRs) are the integral membrane proteins that detect extracellular ligands and mediate signal transduction. Binding of ligands promotes transitions from inactive to active receptor conformations, which are recognized by intracellular effectors. Chemically distinct ligands may trigger different signaling responses by altering dynamics of the receptor. We developed a single-molecule fluorescence system to observe conformational switching of the β2-adrenergic receptor in real-time within a native-like membrane environment. The receptor was covalently labeled with a photostable and environmentally-responsive Cy3 fluorophore at the cytoplasmic end of either transmembrane (TM) helix VI or helix VII. Individual receptor molecules were incorporated in phospholipid nanodiscs, tethered to a microscope cover slip and visualized over time by total internal reflection fluorescence microscopy. We observed spontaneous transitions of TM helices VI and VII between inactive and active conformations, even in the absence of any ligands. Studies of ligands that span a comprehensive range of pharmacological efficacies showed that full agonists shorten the time spent in the inactive conformations and prolong the time in the active conformations, leading to an increased population of active species, while an inverse agonist prolonged the time spent in inactive conformations. These observations provide new insights into the mechanism of GPCR activation and the molecular basis for the variable pharmacological efficacies of different drug molecules.

1759-Plat
Entry from the Lipid Bilayer: A Novel Pathway for Inhibition of a Peptide G-Protein Coupled Receptor by a Lipophilic Small Molecule
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G-protein-coupled receptor (GPCR) crystal structures reveal exquisite details of interactions between amino acids and bound ligands. However, little is known about the pathways by which ligands enter their binding sites. Binding pathways are essential determinants of association and dissociation rates and thus underlie kinetic selectivity, a phenomenon in which a ligand primarily affects a particular receptor despite having similar binding affinity for several closely related receptors. Protease-activated receptor 1 (PAR1) is a GPCR whose endogenous agonist is a tethered, water-soluble peptide generated by thrombin cleavage. By contrast, the PAR1-specific antagonist vorapaxar is a lipophilic small molecule that binds in a pocket almost entirely occluded from extracellular solvent.
Based on temperature-accelerated molecular dynamics simulations, we hypothesize that vorapaxar enters the PAR1 binding pocket from the lipid bilayer between transmembrane helices 6 and 7 (TM6 and TM7). To test this hypothesis, we take a chemical biology approach and synthesize vorapaxar derivatives with alkyl chains extending along the predicted binding pathway. In cell signaling assays, we find that the on-rate for vorapaxar derivatives with increased bulk between TM6 and TM7 is no slower than that of vorapaxar. These data indicate that vorapaxar likely enters the binding pocket from the lipid bilayer, through the cleft between TM6 and TM7. While other groups have reported experimental evidence that retinal binds to rhodopsin through a channel from the lipid bilayer, our study provides the first such data for a ligand binding to a peptide GPCR. Membrane drug entry mechanisms, such as the pathway we describe for vorapaxar binding to PAR1, may be important for understanding kinetic selectivity of lipophilic GPCR ligands and may inform selective drug design.

1760-Plat
Single Molecule Imaging of M_2 Muscarinic Receptors in Live Heart Explants
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TIRF microscopy allows us to visualize individual fluorophores in living cells. We have developed automated algorithms to detect and track thousands of individual molecules and have studied GPCRs (G-protein coupled receptors) tagged with fluorescent ligands or fluorescent proteins in live cultured cells and freshly dissected tissue slices.
We have found that M_1 and M_2 muscarinic receptors diffuse freely at the plasma membrane with their mobility dependent upon both cell-type and temperature. The mobility of M_2 receptors tagged with the fluorescent antagonist (Cy3B-telenzepine) matches the mobility of M_2-GFP molecules imaged under the same conditions. Fluorophore tracking allowed direct observation of transient homo-dimerisation of M_1 and M_2 receptors which we confirmed using two-color imaging. At a membrane receptor density of 2 μm^{-2}, 30% of receptors were dimeric and the dimer lifetime was ~1 s^{-1}.
We have found that only ~10% of isolated primary cardiomyocytes and cells in the mice heart slices expressed M_2 receptors on the detectable level. The receptors undergo unrestricted diffusion and therefore were evenly distributed across the cell membrane. The M_2 density in freshly isolated embryonic cardiomyocytes was ~1 μm^{-2}, increasing at birth to ~3 μm^{-2} and decreasing back to ~1 μm^{-2} after birth. Whilst some M_2 receptors formed reversible dimers the majority were monomeric. The receptor mobility was approximately 4-times faster in freshly dissected heart slices (0.6 μm^2 s^{-1}) than in cultured primary cardiomyocytes. Knowing receptor mobility and density we used Monte Carlo simulations to estimate an encounter rate of 5-10 collisions per second. This may explain the observed electrophysiological latency between the application of acetylcholine and GIRK channel opening.

1761-Plat
The Structural Basis for Lipid a Recognition in the CD14 Innate Immune Co-Receptor
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The CD14 co-receptor is specialized for recognition of bacterial lipopolysaccharide (LPS). On the surface of macrophages and other immune cells, it transfers LPS and its bioactive component lipid A to the MD-2 protein in complex with Toll-Like Receptor 4 (TLR4), and is hence crucial in activating the innate immune system via the TLR4 signalling pathway. In the case of severe infections, lipid A can cause sepsis through over-activation of the immune response, leading to multiple organ failure and death, and has become a major target for anti-septic drugs. Unfortunately, the mechanism by which lipid A is transferred to CD14, and the detailed mode(s) of associated binding, are